– *impurities A, C*: for each impurity, not more than 0.4 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.2 per cent);
– *unspecified impurities*: for each impurity, not more than 0.2 times the area of the principal peak in the chromatogram obtained with reference solution (b) (0.10 per cent);
– *total*: not more than twice the area of the principal peak in the chromatogram obtained with reference solution (b) (1.0 per cent);
– *disregard limit*: area of the principal peak in the chromatogram obtained with reference solution (c) (0.05 per cent); disregard any peak due to the blank (solvent mixture).

Water (*2.5.12*): 3.0 per cent to 4.0 per cent, determined on 0.200 g.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*).

Solvent mixture. Mix equal volumes of *acetonitrile R1* and *water R*.

Test solution. Dissolve 20.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (a). Dissolve 20.0 mg of *spirapril hydrochloride monohydrate CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Reference solution (b). Dissolve 6.0 mg of *spirapril for system suitability CRS* (spirapril spiked with impurity B and impurity D) in a mixture of 2 volumes of *acetonitrile R* and 8 volumes of *water R* and dilute to 20 mL with the same mixture of solvents.

Solution A. Dissolve 4.5 g of *tetramethylammonium hydroxide R* in 900 mL of *water R*, adjust to pH 1.75 with *phosphoric acid R* and add 100 mL of *acetonitrile R1*.

Solution B. Dissolve 4.5 g of *tetramethylammonium hydroxide R* in 400 mL of *water R*, adjust to pH 1.75 with *phosphoric acid R* and add 600 mL of *acetonitrile R1*.

Column:
– *size*: *l* = 0.125 m, Ø = 4.6 mm;
– *stationary phase*: *octadecylsilyl silica gel for chromatography R* (5 µm);
– *temperature*: 70 °C.

Mobile phase: solution A, solution B (45:55 *V/V*).

Flow rate: 2.0 mL/min.

Detection: spectrophotometer at 210 nm.

Injection: 20 µL.

Retention time: spirapril = 1.6 min to 2.9 min; impurity D = about 13 min. Adjust the proportion of solution B in the mobile phase if necessary.

System suitability: reference solution (b):
– *resolution*: minimum 15 between the peaks due to spirapril and impurity D.

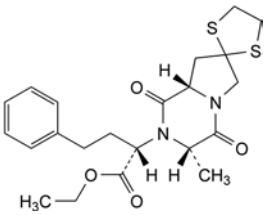
Calculate the percentage content of $C_{22}H_{31}ClN_2O_5S_2$ from the chromatograms obtained with the test solution and reference solution (a) and the declared content of *spirapril hydrochloride monohydrate CRS*.

STORAGE

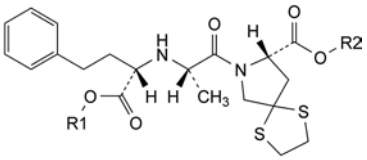
In an airtight container, protected from light.

IMPURITIES

Specified impurities: A, B, C, D.

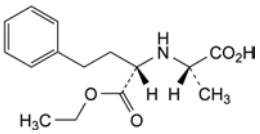


A. ethyl (2*S*)-2-[(3′*S*,8′a*S*)-3′-methyl-1′,4′-dioxohexahydrospiro[1,3-dithiolane-2,7′(6′*H*)-pyrrolo[1,2-*a*]pyrazin]-2′-yl]-4-phenylbutanoate,



B. R1 = R2 = H: (8*S*)-7-[(2*S*)-2-[[(1*S*)-1-carboxy-3-phenylpropyl]amino]propanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylic acid (spiraprilat),

D. R1 = C_2H_5, R2 = $CH(CH_3)_2$: 1-methylethyl (8*S*)-7-[(2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoyl]-1,4-dithia-7-azaspiro[4.4]nonane-8-carboxylate,

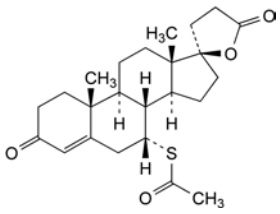


C. (2*S*)-2-[[(1*S*)-1-(ethoxycarbonyl)-3-phenylpropyl]amino]propanoic acid.

01/2011:0688

SPIRONOLACTONE

Spironolactonum



$C_{24}H_{32}O_4S$ M_r 416.6
[52-01-7]

DEFINITION

(2′*R*)-7α-(Acetylsulfanyl)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione.

Content: 97.5 per cent to 102.0 per cent (dried substance).

CHARACTERS

Appearance: white or yellowish-white powder.

Solubility: practically insoluble in water, soluble in ethanol (96 per cent).

It shows polymorphism (*5.9*).

IDENTIFICATION

First identification: A.

Second identification: B, C.

A. Infrared absorption spectrophotometry (*2.2.24*).

Comparison: *spironolactone CRS.*

If the spectra obtained in the solid state shows differences, dissolve the substance to be examined and the reference substance separately in the minimum volume of *methanol R*, evaporate to dryness and record new spectra using the residues.

B. Thin-layer chromatography (*2.2.27*).

Test solution. Dissolve 20 mg of the substance to be examined in *methylene chloride R* and dilute to 10 mL with the same solvent.

Reference solution. Dissolve 20 mg of *spironolactone CRS* in *methylene chloride R* and dilute to 10 mL with the same solvent.

Plate: *TLC silica gel F_{254} plate R.*

Mobile phase: *water R, cyclohexane R, ethyl acetate R* (1:24:75 *V/V/V*).

Application: 5 µL.

Development: over 3/4 of the plate.

Drying: in air.

Detection: examine in ultraviolet light at 254 nm.

Results: the principal spot in the chromatogram obtained with the test solution is similar in position and size to the principal spot in the chromatogram obtained with the reference solution.

C. To about 10 mg add 2 mL of a 50 per cent *V/V* solution of *sulfuric acid R* and shake. An orange solution with an intense yellowish-green fluorescence is produced. Heat the solution gently; the colour becomes deep red and hydrogen sulfide, which blackens *lead acetate paper R*, is evolved. Add the solution to 10 mL of *water R*; a greenish-yellow solution is produced, showing opalescence or a precipitate.

TESTS

Specific optical rotation (*2.2.7*): – 41 to – 46 (dried substance).
Dissolve 0.100 g in *ethanol (96 per cent) R* and dilute to 10.0 mL with the same solvent.

Related substances. Liquid chromatography (*2.2.29*). *Prepare the solutions immediately before use.*

Solvent mixture: *acetonitrile R, water R* (50:50 *V/V*).

Test solution (a). Dissolve 50.0 mg of the substance to be examined in 2.5 mL of *tetrahydrofuran R* and dilute to 25.0 mL with the solvent mixture.

Test solution (b). Dilute 1.0 mL of test solution (a) to 100.0 mL with the solvent mixture.

Reference solution (a). Dilute 1.0 mL of test solution (b) to 10.0 mL with the solvent mixture.

Reference solution (b). Dissolve with the aid of ultrasound the contents of a vial of *spironolactone for system suitability CRS* (containing impurities A, C, D, E and I) in 1.0 mL of the solvent mixture.

Reference solution (c). Dissolve 50.0 mg of *spironolactone CRS* in 2.5 mL of *tetrahydrofuran R* and dilute to 25.0 mL with the solvent mixture. Dilute 1.0 mL of this solution to 100.0 mL with the solvent mixture.

Reference solution (d). Dissolve 5.0 mg of *canrenone CRS* (impurity F) in 2.5 mL of *tetrahydrofuran R* and dilute to 25.0 mL with the solvent mixture. Dilute 3.0 mL of this solution to 100.0 mL with the solvent mixture.

Column:

– *size*: *l* = 0.15 m, Ø = 4.6 mm;
– *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (3 µm);
– *temperature*: 40 °C.

Mobile phase: *acetonitrile R, tetrahydrofuran R, methanol R1, water R* (15:20:425:540 *V/V/V/V*).

Flow rate: 1.0 mL/min.

Detection: spectrophotometer at 254 nm.

Injection: 20 µL of test solution (a) and reference solutions (a), (b) and (d).

Run time: 2.5 times the retention time of spironolactone.

Identification of impurities: use the chromatogram supplied with *spironolactone for system suitability CRS* and the chromatogram obtained with reference solution (b) to identify the peaks due to impurities A, C, D, E and I; use the chromatogram obtained with reference solution (d) to identify the peak due to impurity F.

Relative retention with reference to spironolactone (retention time = about 26 min): impurity A = about 0.95; impurity F = about 1.2; impurity C = about 1.5; impurity D = about 1.6; impurity E = about 1.7; impurity I = about 1.9.

System suitability: reference solution (b):

– *peak-to-valley ratio*: minimum 1.5, where H_p = height above the baseline of the peak due to impurity A and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to spironolactone.

Limits:

– *correction factor*: for the calculation of content, multiply the peak area of impurity F by 2.3;
– *impurity I*: not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.5 per cent);
– *impurities E, F*: for each impurity, not more than 3 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.3 per cent);
– *impurities A, C*: for each impurity, not more than twice the area of the principal peak in the chromatogram obtained with reference solution (a) (0.2 per cent);
– *impurity D*: not more than 1.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.15 per cent);
– *unspecified impurities*: for each impurity, not more than the area of the principal peak in the chromatogram obtained with reference solution (a) (0.10 per cent);
– *total*: not more than 7 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.7 per cent);
– *disregard limit*: 0.5 times the area of the principal peak in the chromatogram obtained with reference solution (a) (0.05 per cent).

Free thiol compounds. To 2.0 g add 20 mL of *water R*, shake for 1 min and filter. To 10 mL of the filtrate add 0.05 mL of *0.01 M iodine* and 0.1 mL of *starch solution R* and mix. A blue colour develops.

Chromium: maximum 50 ppm.

To 0.20 g in a platinum crucible add 1 g of *potassium carbonate R* and 0.3 g of *potassium nitrate R*. Heat gently until fused, and ignite at 600-650 °C until carbon is removed. Cool, dissolve the residue as completely as possible in 10 mL of *water R* with the aid of gentle heat, filter, and dilute to 20 mL with *water R*. To 10 mL of this solution add 0.5 g of *urea R*, and add a 14 per cent *V/V* solution of *sulfuric acid R* until the solution is just acid. When effervescence ceases, add a further 1 mL of the 14 per cent *V/V* solution of *sulfuric acid R*, dilute to 20 mL with *water R* and add 0.5 mL of *diphenylcarbazide solution R*. The solution is not more intensely coloured than a standard prepared by adding 1 mL of a 14 per cent *V/V* solution of *sulfuric acid R* to 0.50 mL of a freshly prepared 28.3 mg/L solution of *potassium dichromate R*, diluting to 20 mL with *water R* and adding 0.5 mL of *diphenylcarbazide solution R*.

Loss on drying (*2.2.32*): maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 105 °C for 3 h.

Sulfated ash (*2.4.14*): maximum 0.1 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in the test for related substances with the following modification.

Injection: test solution (b) and reference solution (c).

Calculate the percentage content of $C_{24}H_{32}O_4S$ from the declared content of *spironolactone CRS*.

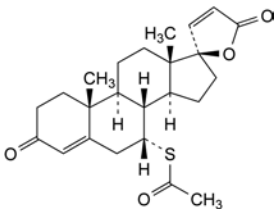
STORAGE

Protected from light.

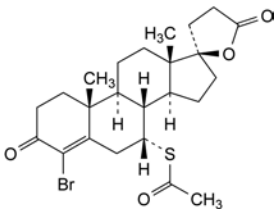
IMPURITIES

Specified impurities: A, C, D, E, F, I.

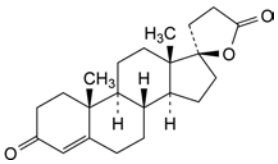
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph *Substances for pharmaceutical use (2034)*. It is therefore not necessary to identify these impurities for demonstration of compliance. See also *5.10. Control of impurities in substances for pharmaceutical use*): *B, G, H.*



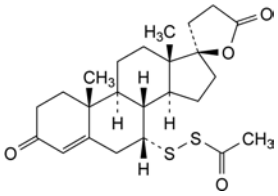
A. (2′*R*)-7α-(acetylsulfanyl)-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione (Δ20-spironolactone),



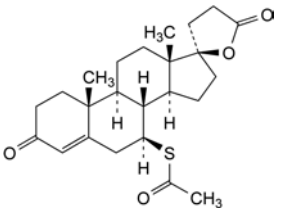
B. (2′*R*)-7α-(acetylsulfanyl)-4-bromo-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione (4-bromospironolactone),



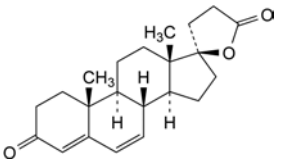
C. (2′*R*)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione (aldone),



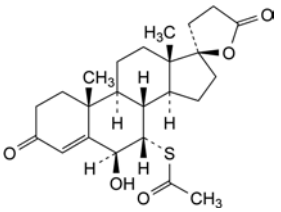
D. (2′*R*)-7α-(acetyldisulfanyl)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione (disulfanyl-spironolactone),



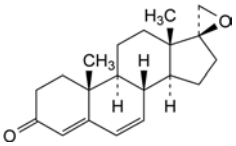
E. (2′*R*)-7β-(acetylsulfanyl)-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione (7β-spironolactone),



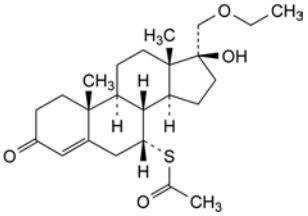
F. (2′*R*)-3′,4′-dihydro-5′*H*-spiro[androst-4,6-diene-17,2′-furan]-3,5′-dione (canrenone),



G. (2′*R*)-7α-(acetylsulfanyl)-6β-hydroxy-3′,4′-dihydro-5′*H*-spiro[androst-4-ene-17,2′-furan]-3,5′-dione (6β-hydroxy-spironolactone),



H. (2′*S*)-spiro[androst-4,6-diene-17,2′-oxiran]-3-one,

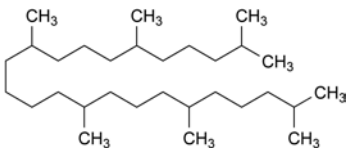


I. *S*-[17α-(ethoxymethyl)-17-hydroxy-3-oxoandrost-4-en-7α-yl] ethanethioate.

01/2008:1630

SQUALANE

Squalanum



$C_{30}H_{62}$ M_r 422.8

[111-01-3]

DEFINITION

2,6,10,15,19,23-Hexamethyltetracosane (perhydrosqualene). It may be of vegetable (unsaponifiable matter of olive oil) or animal (shark liver oil) origin.

Content: 96.0 per cent to 103.0 per cent.